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Numerical Model Study of *In Vivo* Magnetic Nanoparticle Tumor Heating

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Abstract

Iron oxide nanoparticles are currently under investigation as heating agents for hyperthermic treatment of tumors. Major determinants of effective heating include the biodistribution and minimum iron oxide loading required to achieve adequate heating at practically achievable magnetic field strengths. These inter-related criteria ultimately determine the practicality of this approach to tumor treatment. Further, in our experience the currently-used treatment assessment criterion for hyperthermia treatment — Cumulative Equivalent Minutes at 43 °C, CEM₄₃ — provides an inadequate description of the expected treatment effectiveness.

Objectives—Couple numerical models to experimental measurements to study the relative heating effectiveness described by cell death predictions.

Methods—FEM numerical models were applied to increase the understanding of a carefully calibrated series of experiments in mouse mammary adenocarcinoma.

Results—The numerical model results indicate that minimum tumor loadings between approximately 1.3 to 1.8 mg of Fe per cm³ of tumor tissue are required to achieve the experimentally observed temperatures in magnetic field strengths of 32 kA/m (rms) at 162 kHz.

Conclusion—We show that including multiple cell death processes operating in parallel within the numerical models provides valuable perspective on the likelihood of successful treatment.

Significance—We show and believe that these assessment methods are more accurate than a single assessment figure of merit based only on the comparison of thermal histories, such as the CEM method.

Index Terms

Magnetic nanoparticles; Tumor heating; Numerical model analysis; Cell death processes

I. Introduction

Magnetic nanoparticles, typically iron oxide (IONPs) in the form of Fe_3O_4 with some type of bio-compatible coating, such as starch or polyethylene glycol (PEG), are under investigation for tumor treatment, either by direct thermal damage or as an adjunct for other therapies.[1–6] Key to success in this application is obtaining adequate heat in the target tissues.[6–9] It is well understood that tumor vasculature has larger inter-endothelial gaps than most normal tissues resulting in extra-vasation of the IONPs.[10, 11] Subsequently, cells will import IONPs from the extracellular space and cluster them into intracellular endosomes with clusters ranging in size from several hundred to thousands of particles.[12]

However, owing to their small size and the overwhelming influence of local heat transfer, it is not clear that an adequate tumor load of IONP absorbing material can be accumulated to provide sufficient power absorption in practical magnetic fields *in vivo*. [8] In fact, clustering is usually necessary to achieve effective heating.[13] For example, a 10 °C steady-state temperature increase in a single 55 nm diameter nanoparticle requires a volume power density of 5×10^{15} (W/m³), an essentially unachievable power density.[13]

Biodistribution and practically achievable volumetric power density remain to be quantitatively studied *in vivo*. Over the past decade numerical models have become the standard method of assessment of nanoparticle heating in realistic geometries.[13–25] Numerical models enable substantial improved insight compared to analytical approaches. [19, 26, 27]

Consequently, we undertook a series of *in vivo* experiments coupled with realistic Finite Element Method (FEM) numerical models studies to better understand the experimental results. Additionally, we intend to determine practical quantitative engineering design criteria that can be used in treatment planning and assessment. Lv *et al.*[14] claim to have developed such a criterion; but, in fact, have only suggested that the tumor loading should be in the range of 5 – 10 mg_{Fe}/mL of tumor. In a series of *in vitro* studies in four different liver tumor cell lines Yamada *et al.*[28] suggest that tumors 10 mm in diameter require a power density of 1.7 W/g_{tumor} (*i.e.* approximately 1.8 MW/m³) to achieve a steady state temperature of 50 °C, and less for larger size tumors. Etheridge *et al.* [22] studied microliter droplets of ferro-fluid and concluded that (based on heat transfer limitations) treatable tumor volumes must exceed approximately 2 mm in characteristic dimension. We seek a more comprehensive criterion to use for IONP treatment planning.

The only tissue response assessment criterion that has been applied in IONP heating to date has been the Cumulative Equivalent Minutes (CEM₄₃) criterion typically used in hyperthermia studies. CEM₄₃ was applied in IONP heating models by Cheng *et al.*[26], Yamada *et al.*[28], and Mital *et al.*[19] and many others with other heating modalities. Schutt and Haemmerich[16], Soetaert *et al.*[24] and Reis *et al.*[25] used an Arrhenius kinetic model developed by He *et al.*[29] to describe vascular stasis in magnetic fluid hyperthermia. Incidentally, Soetaert *et al.*[24] have apparently used incorrect Arrhenius coefficients in their estimation of vascular stasis.

To date no investigation has directly compared thermal damage and cell death predictions to *in vivo* experimental results; likewise, to date, no investigation has employed Arrhenius kinetic models of cell death, nor investigated the expected response of different cell types to thermal insult in a single heating protocol. In this investigation, we apply improved Arrhenius kinetic models of cell death for four thermally unique cell types[30] to provide an estimate of the spectrum of responses that might be expected for the measured and modeled thermal histories. We will also demonstrate the inadequacy of the single-value CEM₄₃ thermal dose prediction to describe the expected responses.

It must be appreciated that the number and variety of randomizing influences and inherent uncertainties render it impossible to precisely duplicate any *in vivo* experiment. For example, uncertainties and randomizing influences include at least: thermo-physical properties, perfusion spatial distribution, murine physiological responses, IONP spatial distribution, and magnetic field magnitude spatial distribution *vs* mouse positioning. Further, Pennes' model of perfusion,[31] as is implemented in these and all of the other numerical model studies cited, is well known and thoroughly documented to be incorrect. However, more accurate models, such as those by Weinbaum *et al.*[32], Baish *et al.*[33, 34], and others are intractable without substantial amounts of anatomical data, which do not exist except for a very few cases, and certainly not in this case. Some comfort is derived from the observation that the tumor vascular bed geometry is dominated by capillaries, and the Pennes model at least includes perfusion in some sense, and has been used by all of the numerical model studies cited.

II. Methods

A. Experimental Studies

All animal experimentation was conducted under protocols approved by the Dartmouth IACUC in accordance with NIH guidelines.

Bilateral murine mammary adenocarcinoma tumors (MTG-B) were implanted in the fore shoulders of six female C3H mice (The Jackson Laboratory, Bar Harbor, ME) and allowed to develop for two weeks prior to treatment; one to be heated and the other as a the control. Resulting tumor volumes ranged from 250 to 508 mm³ at the time of treatment.

The tumors on one side were injected with BNF IONP, which are composed of Fe₃O₄ crystals (10–20 nm in diameter) coated with a hydroxyethyl starch coating, resulting in a total average hydrodynamic radius of 110 nm (MicroMod GmBH, Rostock, Germany). The tumors were directly injected with 7.5 mg of iron per gram of tumor. Magnetic fluid total injection volumes ranged from 77 to 156 μ l. The IONP were exposed to magnetic fields between 20 and 50 kA/m (rms) at 162 kHz for heating times between 300 and 3,600 s (5 to 60 minutes). The other-side tumor was injected with an equal volume of PBS, and acted as the control tumor.

Transient intra-tumoral temperatures were recorded at 1s intervals at “shallow”, “center” and “tip” locations separated by 3 mm, as sketched in Fig. 1, with optical probes 0.56 mm in diameter (FISO Inc., Quebec, QC, Canada). The center probe was placed as close as

possible to the approximate center of the irregularly shaped tumor. Additionally, rectal, skin surface and several nearby point temperatures were also recorded. The real-time skin surface temperature was recorded with a FLIR Systems (Wilsonville, OR) thermal camera.

Mice were euthanized at 0, 24 hours, or 48 hours post-treatment. The tumor and surrounding tissues were excised and fixed in 10% neutral buffered formalin, routinely processed for 4 μm sections, and stained with Hematoxylin and Eosin (H&E) and Prussian blue stains.

B. Numerical Model Studies

Three-dimensional FEM numerical models were constructed and executed in COMSOL (Comsol, Inc. Burlington MA).

1. Model Space Geometry—The 3D model space consisted of a 2 cm wide (x-axis) by 2.5 cm long (y-axis) by 1 cm thick (z-axis) tissue space, of which the top 0.75 mm represented the skin (Fig. 2). Tumors were represented by 3D ellipsoids (shaded volume in the figure) based on the measured tumor dimensions for the individual experiments and covered with a 0.75mm thick skin subdomain. The simulated tumors were generally centered at the skin surface for the smaller tumors, and above that in the models of larger tumors.

2. Thermal Model—The models assumed an equivalent volumetric heating in the volume power generation term in the Bioheat Equation, Q_{gen} (W/m^3) (spatially adjusted in some cases to better match the measured transient temperatures):

$$\rho_t c_t \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + Q_{\text{gen}} + Q_{\text{met}} + w \rho_t c_b [T - T_a] \quad (1)$$

where: ρ_t = tissue density (kg/m^3), c = specific heat (J/kg), k = thermal conductivity ($\text{W}/\text{m}/\text{K}$), Q_{met} = metabolic heat (W/m^3), w = perfusion ($1/\text{s}$) and T = temperature (K). The thermal properties chosen are representative of the tissues listed (see Table I).

Metabolic heat for muscle was assumed to be similar to the overall resting metabolic rate for humans, approximately $1 (\text{kW}/\text{m}^3)$ [35]. The skin was modeled at 25% of that metabolic rate, and the tumor assumed to be 30% higher than resting muscle. Perfusion values were taken from Diller *et al.* for skin and muscle.[36] The initial tumor perfusion, $3 \times 10^{-3} (1/\text{s})$, was increased to as much as $7 \times 10^{-3} (1/\text{s})$, as required to match experimental results. Two of the experiments displayed the effects of vascular shut down in the transient temperature records, and that feature was added to those models.

The bottom surface, Z_{min} , (Fig. 2) was isothermal at 37°C , and the sides were thermally insulating (zero-flux). The top skin surface was a convective heat transfer boundary with convection coefficient $h = 20 (\text{W}/\text{m}^2/\text{K})$, except in one case as noted. A cooling period of 600 s preceded the heating phase to establish a pre-treatment steady state homeostatic temperature field — separate steady-state solutions verified that steady state had been

achieved by 600 s. The resulting skin temperature of approximately 32 °C at the end of the cooling phase agreed reasonably well with the experimental measurements.

The spatial distribution and magnitude of Q_{gen} were first adjusted somewhat until the experiment maximum transient (or steady state) temperature record from the embedded probes (Fig. 1) was closely approximated by the numerical model result. Where necessary, the perfusion and heat transfer coefficient were then adjusted to improve match to the measurements (*e.g.* Fig. 8).

We chose not to apply Gaussian spatial distributions to the injected nanoparticles, as was suggested by Salloum *et al.*[37], because the measured distributions in our histologic work did not support them.

Volumetric heating in the iron oxide nanoparticles is dominated by Neél relaxation processes in the net magnetic spin moments at 160 kHz.[38] According to the Poynting Power Theorem the volumetric power generation is determined by the imaginary part of the magnetic permeability, $\mu'' \mu_0$ (H/m):[13]

$$Q_{gen} = \omega \mu'' \mu_0 |\mathbf{H}|^2 \quad (2)$$

where: ω = the angular frequency (r/s), and $\mathbf{H}$ = the (rms) vector magnetic field strength (A/m).

The Neél relaxation phenomenon appears as an opening in the $\mathbf{B}$ – $\mathbf{H}$ hysteresis loop, where $\mathbf{B}$ = the magnetic flux density (T). For the BNF-starch mNPs used in this study the hysteresis loop is maximally open at about 14 (kA/m) rms, where the relative $\mu'' = 6$, as in Fig. 3.[13]

The experiments were conducted at 162 kHz in a field strength of between 20 and 50 (kA/m, rms) where the relative imaginary permeability μ'' varies from 5 to about 1.5 (nominally 2.5 at the geometric mean value of 32 kA/m). The maximum achievable volumetric power density in the iron oxide at 32 kA/m was approximately 9×10^9 (W/m³). In all of the experiments, the applied magnetic field strength was kept constant; but, of course, varied spatially as described owing primarily to differences in the elevation of the mouse tumor above the single-turn coil (which had central flux concentrators), as described elsewhere.[6] The nanoparticles have average hydrodynamic diameters of 110 nm. From the nominal IONP dimensions and estimated starch coating thickness the Fe₃O₄ was estimated to occupy between 30 and 43% of the nanoparticle volume, and only a very small fraction of the total tumor volume in each experiment.

The effective tumor loading of the injected nanoparticles cannot be determined by experimental means alone. The numerical model results were therefore used to estimate the tumor loading achieved in each of the experiments by comparing the effective Q_{gen} required to model the temperature record with the maximum achievable heating: effective $Q_{gen} =$ total tumor power/measured tumor volume.

Two of the experiments exhibited evidence of micro-vascular shut down. An attempt to include published vascular shut down models suggested by Brown *et al.* [39] and by He *et al.* [40] did not yield acceptable results. Thus, in those models the micro-vascular perfusion was shut down at the observed time in the experiment using a conditional statement in Comsol.

3. Cell Death Models—The numerical models also included four cell death process predictions based on published data by other investigators. Three of the models were determined from the same cell death assay, propidium iodide uptake in: 1) the AT1 subline of Dunning R3327 rat prostate cells[41], 2) SN12 human renal carcinoma cells[42], and 3) PC3 human prostate carcinoma cells,[43]. The fourth cell line was the classic Chinese Hamster Ovary (CHO) cell data from Sapareto and Dewey's 1978 experiments[44] as improved by Pearce in 2015.[30] This process was loss of clonogenicity in those cells, which is not precisely a cell death assay. However, these experiments were the basis of the CEM₄₃ method, and are therefore classically important.

Propidium iodide (PI) uptake indicates cell death due to severely compromised plasma membranes, so differences among those damage process predictions are either due to differing cellular responses to thermal stress or differing plasma membrane dissolution mechanisms. The fourth CHO "cell death" process was included to indicate the relative sensitivity of the different cell lines to thermal stress.

The cell death model was directly calculated from Arrhenius kinetics, as was appropriate for the AT1 and SN12 cells:

$$\Omega(\tau) = \ln \left\{ \frac{C(0)}{C(\tau)} \right\} = \int_0^\tau A e^{\left[\frac{-E_a}{RT(t)} \right]} dt \quad (3a)$$

$$C(\tau) = e^{-\Omega} \quad (3b)$$

where: $C(\tau)$ = the remaining undamaged or surviving cell fraction, τ = total experiment time (s), T = temperature (K), E_a = the activation energy (J/mole), and A = the frequency factor (s^{-1}) for the damage process (Table II).

The AT1 and SN12 cells are well described by Eqs. (3) alone. However, for the PC3 and CHO cells, it is necessary to add a temperature-dependent time delay to obtain acceptably accurate results.[30] The PC3 cell PI uptake data are well fit by a time delay of:

$$t_d = b - mT = 2703 - 49.6T \quad (4)$$

where: t_d = the delay time (s), and T = the temperature ($^{\circ}\text{C}$). The CHO cells are well fit by a time delay of [45]:

$$t_d = b - mT = 26062 - 555.9T \quad (5)$$

When the model heating time exceeds the required time delay calculated from Eqs. (4) and (5) the PC3 and CHO Arrhenius cell death models commence.

In the numerical models a separate integrating mode was added for each cell death process and all damage process models were disabled below 43 °C. A conditional function was used to: 1) initiate heating after an initial cooling phase, 2) to terminate heating and begin the after-cooling phase, 3) disable the damage process models below 43 °C, and 4) to initiate the PC3 and CHO cell death processes after the delay time elapsed. The local value of the required time delay, t_d , was estimated using Eqs. (4) and (5) in the transient temperature field.

Direct calculation of the time delay by Eqs. (4) and (5) ignores potential cumulative effects of the temperature history; however, for a linear temperature increase no error results from this simplification, while for a square-root of time temperature history the error is only about 20 seconds over a 37 to 60 °C span. Thus, direct calculation is considered adequate in this model series.

The maximum temperature for which a time delay is needed is 54.5 °C for the PC3 cells and 46.9 °C for the CHO cells. The four cell death processes are compared at 50 °C in Fig. 4. At 50 °C the required time delays are 223s for the PC3 cells and 0 for the CHO cells. Note that the PC3 damage curve crosses the SN12 curve after the time delay expires and has a rate of descent faster than the AT1 cells.

The Cumulative Equivalent Minutes at 43 °C (CEM_{43}) assessment of thermal dose was derived from the CHO cell experiment studies, and is the currently-used method in hyperthermia studies.[46, 47] It compares transient thermal histories to an equivalent exposure at 43 °C. In discrete interval form it becomes:

$$CEM_{43} = \sum_{i=1}^N R_{CEM}^{(43-T_i)} \Delta t_i \quad (6)$$

where: R_{CEM} is the time scaling ratio, 43 °C is the reference temperature, and t_i (min) is spent at temperature T_i (°C). For rodent studies R_{CEM} is taken to be 0.45, and approximately 0.5 in human hyperthermia. The actual value of R_{CEM} derived from the CHO Arrhenius coefficients is 0.478.[48]

III. Results

Experiment heating times ranged from 300 to 3600 (s) and maximum temperatures from 39 to 57 °C. Histologic section revealed the variation in the spatial distribution of IONP uptake. Numerical model volume power generation was adjusted for center amplitude and Y-axis variation in order to better represent the indicated experimental biodistribution in some of

the models. In two cases the perfusion was shut down at times indicated by the experimental temperature histories.

In all of the numerical models 600 s were allowed to reach the resting steady state before heating commenced, so the initial temperatures were 32–33 °C, approximating the experiment.

A. Heating for 300 s to 51.5 °C

The experiment in Fig. 5 had the shortest duration in the ensemble, and achieved 51.5 °C after 300 s of heating. Steady state had not been reached at the conclusion of heating (Fig. 5a). The particular tumor in this experiment had ellipsoidal diameter dimensions of 11, 8.5 and 6.3 (height) mm (volume = 307 mm³). Experiment CEM₄₃ values are: Tip = 1033, Center = 77, and Shallow = 1.5 equivalent minutes using the typical rodent time scaling factor $R_{CEM} = 0.45$.

The numerical model (Fig. 5b) provided a reasonable match when the tumor center was set to $Q_{gen} = 0.98$ (MW/m³) — a linear gradient about the center of the tumor was added to match the experiment (slope = 124.7 W/m⁴); and $Q_{gen} = 0$ for $y > 2.5$ mm. A total of 0.270 W was coupled to the 307 mm³ tumor ellipsoid. After cooling for 600s the temperature in the tumor (34 °C) was slightly higher than the surrounding skin (33.7 °C) due to its slightly higher metabolic heat (Table I).

The second phase, tumor heating, applied $Q_{gen}(y)$ for the heating duration, 300s, and 200 s of cooling followed. The cooling transient temperature record matched the experiment with acceptable accuracy (Fig. 5), indicating that the assumed heat transfer parameters were reasonable.

1. Temperature Predictions—As is the usual case, there are a few differences between the experiment and numerical model. For example, in Fig. 5 the shallow temperature probe location in the numerical model reached 43.1 °C (and CEM₄₃ = 0.65), and the measured shallow probe reached 43.3 °C. The center sensor reached 50.3 °C (and CEM₄₃ = 550), compared to experiment values of 48 °C and CEM₄₃ = 77.

[49]The tip sensor final temperature, 51.6 °C, is very close to the experiment value, 51.5 °C, however the initial rate of temperature rise is slightly higher, so the CEM₄₃ value, 1,491, is larger than for the experiment, 1,033. The model - experiment tip temperature difference was a maximum of 2.8 °C at 20 s of heating, and decreased to 0.1 °C at 300 s.

In this experiment the gross histologic sections, Fig. 6 (parallel to the skin surface, that is, the x–y plane), show an IONP distribution weighted anteriorly, *i.e.* near the tip and center locations and virtually devoid in the posterior region, explaining why the volume power density was specified as described. The tumor was harvested approximately 5 min. after heating so that the IONPs could be observed before they dispersed.

2. Cell Death Predictions—The SN12 cells are much more thermally-robust than the AT1, PC3, and CHO cells. In Fig. 7, the cell death predictions corresponding to the

experiment described in Figs. 5 and 6 and the model results (Table III) bear this out. The kinetic nature of the damage process development is also evident in the differences between AT1 and SN12 cell death at differing heating durations. In this model the PC3 cell model predicts a maximum of 88% death (Fig. 7c), the same as the AT1 cells (also 88%, not shown), and the SN12 model a maximum of 11% (Fig. 7d). The CHO 99% cell death region (Fig. 7b) encompasses approximately 60 to 70% of the tumor volume.

B. Heating for 900s to 51 °C with Vascular Shut Down

Two of the experiments showed evidence of vascular shutdown — a rapid temperature increase in the final stages of heating. One example is shown in Fig. 8a, wherein the final maximum tumor temperature, 51.3 °C, only occurred in the final few seconds of heating.

Based on a time scaling ratio of $R_{CEM} = 0.45$ the corresponding CEM_{43} values were: Tip = 1,192, Center = 827, and Shallow = 19 equivalent minutes. In the corresponding FEM model, it was necessary to increase the tumor perfusion to 0.007 (1/s), comparable to the high end of the range for skeletal muscle,[36] to obtain the appropriate end term temperature spike after perfusion shut down. The applied uniform volume power deposition term was $Q_{gen} = 7 \times 10^5$ (W/m³) in this model. The total applied power coupled to the 310 mm³ tumor was 0.243 W.

1. Temperature Predictions—In the numerical model, Fig. 8b, the tumor perfusion was shut down at 600 s and a similar late term heating spike to 50.9 °C occurred (compare to 51.3 °C). The maximum deviation between the model and experimental temperatures was +1.9 °C at $t = 20$ s. At the end of heating the model underestimated the tip temperature by 0.4 °C. The numerical model CEM values are: Tip = 1,013 (compare to 1,192), Center = 1,490 (compare to 827), and Shallow = 219 equivalent minutes (compare to 19). The model has over-predicted the center and shallow locations, and slightly under-predicted the tip location transient temperatures.

In this experiment the IONPs at the level of the section were concentrated in the lower right quadrant (Fig. 9b), which correlates with the location of the dead cells (Fig. 9a), as would be expected. The tumor was harvested approximately 5 min. after heating so that the IONPs could be observed before they dispersed.

2. Cell Death Predictions—This FEM model tumor center was located 2 mm above the skin surface in this model, consequently the plots in Fig. 10 do not show surrounding skin. In Fig. 10a the temperature color scale spans 42 to 51 °C. In Fig. 10b the CHO 99% cell death boundary includes the entire tumor volume. In contrast, the maximum cell death prediction for PC3 cells is 87.1% (Fig. 10c), and is nearly identical to the AT1 cells (91.8%, not shown).

At 45 °C the time delay for the PC3 cells is 471 s, approximately half of the heating time — that is, the PC3 cell death model likely did not commence until about the same time as vascular shut down was enforced. The maximum death probability for the SN12 cells is 10.1 % (Fig. 10d).

C. Heating for 600s to 57 °C

The highest temperature result in the ensemble reached a maximum measured temperature of 57.4 °C in the center location (Fig. 11a). Experiment CEM₄₃ values are: Tip = 20,494, Center = 227,311, and Shallow = 12,688 equivalent minutes.

This numerical model did not require alterations in the uniform spatial distribution of mNPs to obtain an acceptable result (Fig. 11); however, the temperature-sensing catheter position was moved 0.5 mm caudally (–y axis) to simulate a slightly higher measured tip temperature as compared to the shallow location. The applied uniform volume generation in the tumor was $Q_{gen} = 1.1$ (MW/m³), and the surface convection coefficient, h , was reduced to 7 (W/m² K). The total power coupled to the 329 mm³ tumor was 0.361 W.

1. Transient Temperature Predictions—The transient developments are illustrated in Fig. 11 for comparison. The comparable numerical model reached 57.5 °C at the center location after 600 s of heating. The center point temperature in the numerical model was within 0.5 °C of the experiment values everywhere during heating, except at the beginning and end where some edge jitter is expected. The numerical model has slightly over-estimated the tip and shallow location transient temperatures, while fairly representing the center location. The total coupled power was 0.361 W.

2. Cell Death Predictions—Histologic results are summarized in Fig. 12, arranged comparably to those in Fig. 6. The mid-tumor tissue cross-section reveals cell death (Fig. 12a) corresponding to mNP concentrations near the tumor center and in the upper right quadrant (Fig. 12b). In Fig. 12c, the 40X (original magnification) close up taken near the upper quadrant cell death boundary illustrates the disruption in the cell bodies, the stroma, and local vasculature, as indicated by the extra vascular red blood cells. This histologic section demonstrates the sharp necrosis – viability demarcation possible with IONP heating. It also points out the need to understand the relationship of iron concentration and distribution prior to delivery of tissue heating.

The numerical model CEM₄₃ values are: Tip = 79,966 (compare to 20,494 for the experiment), Center = 255,712 (compare to 227,311), and Shallow = 24,634 (compare to 12,688) equivalent minutes.

A center plane temperature field for the model is shown in Fig. 13a. Model cell death predictions show complete cell death for the AT1 cells (Fig. 13b). The 99% isosurface in this figure encloses the entire tumor volume and is open at the top because the skin is included within the AT1 isosurface. The CHO cells show a similar response (not plotted). We estimate that the AT1 (and CHO) cell death regions occupy at least 120% of the tumor volume. The cell death prediction for PC3 cells has a maximum of 99% over 80 to 90% of the tumor volume. In Fig. 13d The SN12 cell death prediction (same color scale) has a maximum probability of 82.6% at the tumor center. At 57 °C the CHO cells are expected to have 99% death probability for $\tau < 0.2$ s, the AT1 cells at $\tau = 80$ s, the PC3 cells at 95 s (no time delay), and the SN12 cells at 965 s.

D. Discussion of the Results

The model conditions were adjusted so that the maximum transient temperatures matched the experiments as closely as could be achieved. FEM numerical model volume average power densities (Q_{gen}) were selected to provide the best match to the steady state final temperatures in order to provide an estimate of the effective power coupled to tumor tissue. The total power required to simulate the experiment was calculated by subdomain integration in the FEM model space.

1. Uncertainties in the Model Calculations—Of course, there are deviations between the two types of records owing to the multiplicity of uncertain physical properties, effects of perfusion, thermal boundary conditions, magnetic field spatial variations, and biodistribution of the IONPs. Even when injected directly into a tumor some of the ferrofluid does not penetrate into the tumor tissues (intra-cellular and extra-cellular) but is carried by perfusion (blood and lymph) to other parts of the body. See for example: Attaluri et al.[49] Consequently, the maximum deviations between the model and experiment temperatures typically occurred during early heating and post-heat cooling. The maximum temperature was chosen as the matching variable because the cell death models are hyperbolically dependent on temperature in the exponent and extremely sensitive to small temperature differences.

2. Required Model Powers—The volume power densities, Q_{gen} , required to simulate the measured transient temperatures ranged from 0.7 to 1.1 (MW/m³). In calculating the power density by Eq. (2) the effective value of the imaginary permeability includes the volume fraction, V_f of magnetic material, Eq. (7).

$$Q_{\text{gen}} = (2\pi f) (\mu'' \mu_0 V_f) |\mathbf{H}|^2 \quad (7)$$

where: f = frequency (162 kHz), and μ_0 = free space permeability, 1.26×10^{-6} (Hy/m).

3. Estimates of Tumor Loading—The results summarized in Table IV are listed in sequence of the maximum experimental temperature, the same sequence as in Table III. The values of volume fraction, V_f were determined by comparing the total numerical model power to the measured tumor volume in Eq. (7) at the nominal magnetic field strength of 32 (kA/m), where the relative $\mu_r'' = 2.5$. V_f was then used to estimate the equivalent mass of iron in the tumor assuming an iron oxide density of 5,242 (kg/m³). The numerical model effective volume power densities at 32 (kA/m) ranged from 0.78 to 1.10 (MW/m³).

The effect of uncertainty in the magnetic field strength is significant. At the nominal applied external magnetic field strength of 32 (kA/m) (the geometric mean of 20 – 50) a nominal $Q_{\text{gen}} = 1$ (MW/m³) requires a volume fraction of the BNF IONPs of $V_f = 313 \times 10^{-6}$, or a mass fraction of approximately $m_f = 1.64$ (mg_{Fe3O4}/cm³). At $|\mathbf{H}| = 20$ (kA/m) (relative $\mu_r'' = 4$) the required mass fraction would be $m_f = 2.56$ (mg_{Fe3O4}/cm³), while at 50 (kA/m) (relative $\mu_r'' \approx 1.5$) the required mass fraction would be $m_f = 1.09$ (mg_{Fe3O4}/cm³).

4. Cell Death Predictions—The impact of the kinetic nature of cell death processes is evident in the trends over the range of heating times. The CHO cells are extremely thermally sensitive compared to all of the other cell types.

In all of the heating models the AT1 and PC3 cells show essentially the same cell death probability, despite the difference in their activation energies, 244.8 and 222.2 (kJ/mole), respectively. This is due to the effect of the time delay required for the PC3 cells — all of the heating times are long enough to reach the region of similarity between these two processes, as shown in Fig. 4. The SN12 cells are remarkably robust against thermal stress compared to the other three cell types.

In all cases the CHO cell and CEM predictions suggest that the heat treatment was likely quite successful. However, focusing only on the most sensitive cell type gives an inappropriately optimistic picture of likely success, in our opinion. The SN12 cells were hardly affected until a high temperature (56 – 57 °C) was sustained for 100s of seconds, and even then only 83% cell kill was predicted. The common clinical wisdom is that successful treatments should achieve a 2-log₁₀ decrease in viable cells, *i.e.* < 1% survival. At 43 °C the SN12 cells would require 1.56×10^5 s (43.3 hours) to show such a decrease, illustrating the limitations of the CEM₄₃ criterion.

In 2003 He and Bischof[50] plotted $\ln\{A\}$ vs. E_a for Arrhenius coefficients determined independently by multiple research groups over several decades and showed that they all plot very closely to a single empirical line: $\ln\{A\} = 0.38 E_a \text{ (kJ/mole)} - 9.36$. The rates of cell death accumulation at 50 °C (Fig. 4) appear not to be monotonic with respect to the activation energies, E_a (Table II), as indeed they should be according to the He-Bischof line. [50] In fact, at 50 °C the rates of damage accumulation, $d\Omega/dt$, for the AT1 and PC3 cells are virtually identical — $8.04 \times 10^{-3} \text{ (s}^{-1}\text{)}$ and $8.22 \times 10^{-3} \text{ (s}^{-1}\text{)}$, respectively, whereas the He-Bischof line values would predict 5.8×10^{-4} and 4.9×10^{-4} , respectively. This factor of 10 discrepancy between the predictions of the He-Bischof line and published coefficients is caused by the extreme sensitivity of the damage rate prediction to small uncertainties in the relative coefficients. Both sets of coefficients from Table II plot quite closely to the He-Bischof line (actually, very slightly above it), but result in a non-monotonic progression in damage rate in practice, presumably owing to uncertainties in the experimental data.

IV. Conclusion

The numerical model study has significantly improved our understanding of the experiment series and identified several important underlying phenomena. The superficial locations and small size of tumors mean that surface heat transfer is a governing thermal phenomenon: Most of the experiments resulted in steady-state temperature rises in which Q_{gen} was balanced by local heat transfer. Successful heating of superficial tumors in the size range of 5 to 10 mm in characteristic dimension to temperatures in excess of 50 °C requires an effective tumor IONP loading in excess of approximately $1.25 \text{ mg}_{\text{Fe}}/\text{cm}^3$ at the nominal magnetic field strength of 32 (kA/m rms), but over the range of measured magnetic field strengths this lower limit value varies from about 1 to 3 $\text{mg}_{\text{Fe}}/\text{cm}^3$. This is a significant

treatment design parameter that establishes a useful treatment-planning criterion for IONP biodistribution.

The results also reveal the differing nature of “success” as measured by thermal damage kinetics. That is, a single assessment criterion, such as CEM_{43} , can be substantially misleading since it masks the differences among cell types and the relative kinetics of their responses to thermal insult. While the CEM values were quite high in the experiments, the predicted cell death success rate was substantially lower than would be expected from CEM alone for most of the cell types. Three of the four damage processes in the numerical models were chosen because they result from the same cell death assay — propidium iodide uptake. The three different cell types respond differently to the same heating history.

Realistic treatment planning should incorporate some analysis of the range of responses to be expected and the effect of varying kinetics among cell types. A more realistic picture of likely success is realized by including maximum and minimum thermal sensitivity damage/cell death processes.

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Fig. 1.
Sketch of transient intra-tumoral temperature probe placements at shallow, center and tip locations.

Fig. 2.

FEM 3D model space. Ellipsoidal tumor embedded just below skin surface (shaded). The Y-axis is oriented to be along the track of the point temperature sensors in Fig. 1. The bottom surface, $Z_{\min}$, is isothermal at 37 C, the sides are adiabatic, and the top surface is a convection boundary.

Fig. 3. Imaginary part of the relative magnetic permeability is maximal at about 14 (kA/m) at 150 kHz.[13]

Fig. 4.
Comparison of the four cell death models at 50 °C.

(a)

(b)

Fig. 5.

a) Measured transient temperatures on the embedded optical temperature linear probe at “shallow”, “center” and “tip” locations. The maximum temperature near the tumor center was 48 °C in the experiment, and 51.5 °C at the tip. b) FEM numerical model transient temperatures at similar locations in the simulated tumor. The maximum temperature in the model tumor ellipsoid reached 51.6 °C.

Fig. 6.

The histologic sample was collected 5 minutes after heating. The tumor measures 7 mm in width. a) Hematoxylin and Eosin (H&E) stain indicates thermally damaged cells (irregular blue nuclei and indiscrete pink staining) in the midst of cells with intact nuclei (blue) surrounded by regular amounts of cytoplasm (pink); bar = 5 mm. b) Prussian Blue stain reveals IONP distribution (blue) weighted toward the tip and center probe locations; bar = 5 mm. c) 40X (original magnification) H&E micrograph of region at the boundary of live cells (left) and disrupted dead and dying cells (right) near tip temperature sensor; bar = 50 μm. Some red blood cells are visible in the blood vessel (left side).

Fig. 7.

Predictions of the temperature field and cell death from the FEM numerical model. The tumor and skin subdomains are outlined in the figures. a) Mid-plane (x-y) cross-section temperature field at the end of heating; color scale 34 (blue) to 52 °C (red) (upper center thermometer scale). b) CHO cell death calculation from 0% (blue) to 100% (red) (lower center thermometer scale) with an isosurface at 99% (gray) that covers approximately 90% of the tumor volume. The isosurface is open at the top because it intersects the skin surface. The CHO cell model result is nearly indistinguishable from the AT1 cells except that it is not open at the skin surface owing to the time delay required in the CHO calculation. c) The cell death prediction for PC3 cells has a maximum probability of 88.0% (same color scale). d) The SN12 cell death prediction has a maximum death probability of 11%. Results for the AT1 cells are nearly identical to those of the SN12 cells and are not plotted here.

(a)

(b)

Fig. 8. Temperature locations: Tip = solid line, Center = long dashes, Shallow = short dashes. a) Measured transient temperatures ($T_{\max} = 51.3^{\circ}\text{C}$) and b) FEM numerical model transient temperatures ($T_{\max} = 50.9^{\circ}\text{C}$). The late term spike in the experiment is evidence of vascular shutdown.

Fig. 9.

The histologic sample was collected 5 minutes after heating. The tumor measures 9 mm in width. a) Hematoxylin and Eosin (H&E) stain indicates the tumor located centrally in the section (purple). Large pink areas within the tumor primarily represent focal necrosis; bar = 2 mm. b) Prussian Blue stain reveals IONP distribution (blue) concentrated in a small region in a near center region of the tumor; bar = 2 mm. c) This photomicrograph represents the boundary of viable and heated necrotic tissue. The light brown staining in the center lower right is likely the iron residue following IONP heating. The boundary between tissue viability and necrosis is sharp; bar = 50 μm, H&E 40X.

Fig. 10.

Predictions of the temperature field and cell death from the FEM numerical model described in Fig. 8b. a) Coronal (x-y) temperature field at the end of heating; color scale 42 (blue) to 51 °C (red) (upper center thermometer). b) Predicted CHO cell death is 100% for ~95% of the tumor volume (color scale 0 to 100%, lower center thermometer), isosurface (gray) at 99%. c) The cell death prediction for PC3 cells, maximum probability = 87.1% at the tumor center (color scale 0–100%). d) The SN12 cell death prediction has a maximum death probability of 10.1% at the tumor center. Results for the AT1 cells (maximum 91.8%) are nearly identical to those of the PC3 cells and are not plotted.

(a)

(b)

Fig. 11.

a) Measured transient temperatures (center $T_{\max} = 57.4$ °C) and b) FEM numerical model temperatures (center $T_{\max} = 57.8$ °C).

Fig. 12.

The histologic sample was collected immediately after heating. The tumor measured 8 mm in width. Figures (a) and (b) represent Hematoxylin and Eosin (morphology analysis) and Prussian Blue (iron analysis), respectively; bar = 5 mm. The iron demonstrated in Fig. b corresponds very closely to the necrotic regions (homogeneous pink) seen in Fig. (a). Fig. (c) is a 40X photomicrograph demonstrating necrosis in the lower left quadrant and viable tumor in the upper right quadrant; bar = 50 μm. Highly perfused/congested blood vessels (bv) are visible in the upper region.

Fig. 13.

Predictions of the temperature field and cell death from the FEM numerical model (Fig. 11). The tumor and skin subdomains are outlined in the figures. a) Coronal (x-y) cross-section temperature field at the end of heating, $T_{\max} = 57.5$ °C; color scale 36 (blue) to 57.5 °C (red) (upper center thermometer). b) The predicted AT1 cell death calculation — color scale 0% (blue) to 100% (red) (lower center thermometer) — and isosurface at 99% cell death (gray). c) The cell death prediction for PC3 cells (same color scale) has a maximum of 100% at the tumor center and isosurface at 99% cell death (gray). d) The SN12 cell death prediction (same color scale) has a maximum probability of 82.6% at the tumor center.

Table I

Thermal Model Parameters

Tissue	k	ρ	c	Q_{met}	w
Skin	0.3	700	3700	250	0.88×10^{-3}
Muscle	0.5	1050	3700	1000	1.75×10^{-3}
Tumor	0.4	900	3700	1300	Variable
Blood		1050	4186		

Table II

Damage Process Parameters

Process	E_a (kJ/mole)	A (1/s)	m	b
AT1 cells	244.8	2.99×10^{37}		
SN12 cells	314.9	3.153×10^{47}		
PC3 cells	222.2	6.75×10^{33}	49.6	2703
CHO cells	627.5	7.254×10^{100}	555.9	26062

Table III**FEM Numerical Model Results**

Model Number	Figs.	T _{max} Tumor (°C)	Heating Time (s)	Tip	Center	Shallow	CHO Cell Death % (Vol)	AT1 Cell Death %	SNI2 Cell Death %	PC3 Cell Death % (Vol.)
1	11 – 13	57.5	600	20,494	227,311	12,688	100 (120)	100 (100)	82.6	100 (90)
2 ¹	N/A	54.6 ¹	1300	3,674	20,555	48,107	100 (100)	100 (90)	73.5	100 (80)
3	8 – 10	50.9	900	1,192	827	19	100 (95)	94.2	11.5	91.2
4	5 – 7	51.6	300	1,033	77	1.5	100 (90)	88.2	11	88.3
		40	3600	0.40	0.34	0				
		39	1800	0	0.13	0.11			Not Modeled	

¹ Tumor perfusion shut down at 700s; the majority of the experiment was at 46 °C and spiked to 54.6 at the end (similar to Fig. 8). At 46 °C the time delay for the PC3 cells is 421 s.

Table IV

Mouse Experiment and FEM Numerical Model Comparison

Experiment							Numerical Model		
Tumor Vol. (μL)	Fe (mg)	Max. P _{tot} (W)	Ideal Q _{gen} Max (MW/m ³)	T _{max} (C)	P _{tot} Model (W)	Effective Q _{gen} (MW/m ³)	Estimated Tumor Load (mg _{Fe} /cm ³)		
329	2.45	4.04	6.1	57.4	0.36	1.10	1.76		
508	3.81	6.3	6.2	54.6	0.41	0.80	1.28		
310	2.33	3.84	6.1	51.3	0.24	0.78	1.25		
307	2.3	3.8	6.2	51.5	0.27	0.88	1.41		
250	4.62	7.62	15.3	40				Not Modeled	
302	2.27	3.75	6.1	39					